selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal . These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, Molec. CeIZ. Biol. 3:280(1983).

This invention provides for an oligonucleotide comprising a nucleic acid having the sequence as set forth in Figure 6, including mutants, and variants thereof.

In addition, this invention provides a primer comprising the nucleic acid having the sequence as set forth in Figure 7, including mutants, and variants thereof.

Oligonucleotides which are complementary may be obtained as follows: The polymerase chain reaction is then carried out using the two primers. See *PCR Protocols: A Guide to Methods and Applications* [74]. Following PCR amplification, the PCR-amplified regions of a viral DNA can be tested for their ability to hybridize to the three specific nucleic acid probes listed above. Alternatively, hybridization of a viral DNA to the above nucleic acid probes can be performed by a Southern blot procedure without viral DNA amplification and under stringent hybridization conditions as described herein. Oligonucleotides for use as probes or PCR primers are chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage and Carruthers [19] using an automated synthesizer, as described in Needham-VanDevanter [69]. Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC

as described in Pearson, J.D. and Regnier, F.E. [75A]. The sequence of the synthetic oligonucleotide can be verified using the chemical degradation method of Maxam, A.M. and Gilbert, W. [63].

High stringent hybridization conditions are selected at about 5? C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60?C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents, ie. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. For Example high stringency may be attained for example by overnight hybridization at about 68?C in a 6x SSC solution, washing at room temperature with 6x SSC solution, followed by washing at about 68?C in a 6x SSC in a 0.6x SSX solution.

Hybridization with moderate stringency may be attained for example by: 1) filter pre-hybridizing and hybridizing with a solution of 3x sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at Ph 7.5, 5x Denhardt's solution; 2.) pre-hybridization at 37?C for 4 hours; 3) hybridization at 37?C with amount of labelled probe equal to 3,000,000 cpm total for 16 hours; 4) wash in 2x SSC and 0.1% SDS solution; 5) wash 4x for

1 minute each at room temperature at 4x at 60?C for 30 minutes each; and 6) dry and expose to film.

The phrase "selectively hybridizing to" refers to a nucleic acid probe that hybridizes, duplexes or binds only to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total cellular DNA or RNA. By selectively hybridizing it is meant that a probe binds to a given target in a manner that is detectable in a different manner from non-target sequence under high stringency conditions of hybridization. in a different "Complementary" or "target" nucleic acid sequences refer to those nucleic acid sequences which selectively hybridize to a nucleic acid probe. Proper annealing conditions depend, for example, upon a probe's length, base composition, and the number of mismatches and their position on the probe, and must often be determined empirically. For discussions of nucleic acid probe design and annealing conditions, see, for example, Sambrook *et al.*, [81] or Ausubel, F., *et al.*, [8].

It will be readily understood by those skilled in the art and it is intended here, that when reference is made to particular sequence listings, such reference includes sequences which substantially correspond to its complementary sequence and those described including allowances for minor sequencing errors, single base changes, deletions, substitutions and the like, such that any such sequence variation corresponds to the nucleic acid sequence of the pathogenic organism or disease marker to which the relevant sequence listing relates.

II Methods of creating phage display libraries:

In one embodiment, the nucleic acids are derived from pools of blood. The method provides the generation of diverse libraries. The cells are obtained from a human being or beings. In another embodiment the cells, are obtained from a nonhuman vertebrate species.

This invention provides a method for creating a phage display chimeric TCR/Ig reagent comprising the steps of:: obtaining a sample of cells; mRNA preparation; reverse transcribing mRNA of the cell population into cDNA sequences of T-cell receptor and immunoglobulin; amplifying the cDNA, providing nucleic acid expression vectors which are capable of being packaged; cloning the population of DNA fragments into expression vectors; combining (i) a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population of the TCR-cell receptor elements with (ii) a genetically diverse repertoire of nucleic acid sequences which encodes a unique or genetically diverse population of the immunoglobulin elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR and antibody polypeptide; expressing said library from said vectors in recombinant host organism cells, each of the said polypeptide chain components being expressed as a recombinant chimeric protein on its own or as part of phage particles which are components of the library, also with the property of binding specifically to a target molecule of interest; selecting from said expressed library by binding to a target molecule a unique or restricted population of said reagents binding specificity, thereby producing a recombinant chimeric TCR/Ig reagent.

This invention provides a method for creating a phage display T-cell receptor reagent comprising the steps of: obtaining a sample of cells; mRNA preparation; reverse transcribing mRNA of the cell population into cDNA sequences of T-cell receptor; amplifying the cDNA; providing nucleic acid expression vectors which are

capable of being packaged; cloning the population of DNA fragments into expression vectors; combining a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population of the TCR-cell receptor elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR polypeptide; expressing said library from said vectors in recombinant host organism cells, said polypeptide chain components being expressed as a recombinant TCR protein on its own or or as part of phage particles which are components of the library, also with the property of binding specifically to a target molecule of interest; selecting from said expressed library by binding to a target molecule of interest a unique or restricted population of said reagents binding specificity, thereby producing a recombinant TCR reagent.

PCR reaction conditions should be chosen which optimize amplified product yield and specificity, and, additionally, produce amplified products of lengths which may be resolved utilizing standard gel electrophoresis techniques. Such reaction conditions are well known to those of skill in the art, and important reaction parameters include, for example, length and nucleotide sequence of oligonucleotide primers as discussed above, and annealing and elongation step temperatures and reaction times.

III. <u>Methods for selection of recombinant reagent</u>:

This invention provides a method for selecting recombinant reagents directed against a molecular target, said method comprising: contacting the phage display chimeric library or the phage display TCR library as hereinabove described, with the target molecule so as to

form a complex, dissociating the specifically bound phage from the complex; amplifying the rescued phage in a bacterial host; repeating binding, dissociation and amplification steps; isolating the bound phage thereby selecting said library against a target molecule. In another embodiment the method further comprises characterizing the selected phage particles and their respective reagents.

As used herein, a "target" or "ligand" is a molecule that can bind a recombinant TCR protein or a chimeric recombinant reagent according to the invented method. A target as used herein, is any composition: molecule, a complex, a nucleic acid sequence, a polypeptide, peptide fragment or any composition that can be assayed for its ability to function in given capacity or compound. The target molecule perhaps synthetic, recombinant or biological sample derived for e.g. from body fluid such as serum or urine, part of organ or cells which conceivably derived from a destinct MHC subtype. The cell pupulation may be selected from the group consisting a population of eukariotic cells, such as murine cells, ovine cells, porcine cells, primate cells, human cells, plant cells transformed cells, fused cells and combinations thereof.

The target molecule may be coupled to a solid-phase support, e.g., nitrocellulose, nylon, column packing materials (e.g., Sepharose beads), magnetic beads, glass wool, plastic, metal, polymer gels, cells, or other substrates. Such supports may take the form, for example, of beads, wells, dipsticks, or membranes.

By "solid phase support or carrier" is intended any support capable of binding an target molecules, phage

particle or reagent. Well- known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros (?), and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding target molecules or phage or reagents, or will be able to ascertain the same by use of routine experimentation.

IV. Methods for screening of recombinant reagent

Screening assays can be conducted in a variety of ways which are known to those skilled in the art. The principle assays used to identify compounds that bind to the component parts of library involves binding the target molecule to a support, such as microtiter plates, nitrocellulose, or other solid support that is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable media, e.g. buffers followed by treatment with, the selected component of the libraries, or reagents for a time sufficient to interact and bind, thus forming a complex. After washing away unbound phage particles or reagent, bound material representing the binding partner of a target molecule can be detected by number of ways. Where

the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the previously non-immobilized component.e.g anti-M13 HRP antibodies.

This invention provides a method of detecting a reagent of the recombinant phage library as described above. As provided herein, the junctional regions between the variable domain of the single chain TCR/Ig chimera or single chain TCR contains Cα-TCR or Cβ-TCR N-terminal domain-derived peptides joint to $(Gly_4Ser)_3$ peptide linker. The combination of $(Gly_4Ser)_3$ and Cα-TCR or Cβ-TCR N-terminal domain-derived peptides provide novel epitopes for reagents like Ab, which can recognize them with high specificity and affinity. In addition, Cα-TCR or Cβ-TCR sequences at the C terminal end of the single chain TCR/Ig chimera or single chain TCR provide with novel tag for additional epitopes for reagents like Ab. Bound phage or reagents can therafter analyzed for e.g. amino acid sequence by well-known methods. Reagent so identified can be produced using recombinant DNA technology.

Likewise, a bioluminescent compound may be used to label the target molecule phage or reagents of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is

determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

"Specifically binding to " or "specifically immunoreactive with", when referring to the recombinant reagent contenplated in the present invention refers to the binding of a molecular target or a cell to the recombinant reagent as provided herein. A variety of immunoassay formats may be used to select recombinant reagents specifically immunoreactive with a particular molecular target. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane [32] for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

The target molecule phage or reagents may be labelled with a detectable marker including, but not limited to: a radioactive label, or non redioactive isoltopic lable, a colorimetric, a luminescent, or a fluorescent marker, or heavy metal. Isotopic labels include, but are not limited to: ^{3}H, ^{14}C, ^{32}P, ^{33}P; ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{59}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re, as well as ^{55}Mn, ^{56}Fe. Fluorescent markers include but are not limited to: fluorescein, isothiocyanate, a rhodamine, a phycoerythrin, a phycocyanin, an allophycocyanin, O-phthaldehyde, a fluorescamine, etc.) Other labels may include peridinin chlorophyll protein (PerCP), chemiluminescent labels, enzyme labels (e.g., alkaline phosphatase, horse radish peroxidase, etc.), protein labels, and labels useful in radioimaging and

radioimmunoimaging. Colorimetric markers include, but are not limited to: biotin, and digoxigenin.

Since specific enzymes may be coupled to other molecules by covalent links, the possibility also exists that they might be used as labels for the production of tracer materials. Suitable enzymes include alkaline phosphatase, beta-galactosidase, glucose-6-phosphate dehydrogenase, maleat dehydrogenase, and peroxidase. Two principal types of enzyme immunoassay are the enzyme-linked immunosorbent assay (ELISA), and the homogeneous enzyme immunoassay, also known as enzyme-multiplied immunoassay (EMIT, Syva Corporation, Palo Alto, CA). In the ELISA system, separation may be achieved, for example, by the use of antibodies coupled to a solid phase. The EMIT system depends on deactivation of the enzyme in the tracer-antibody complex; the activity can thus be measured without the need for a separation step.

Additionally, chemiluminescent compounds may be used as labels. Typical chemiluminescent compounds include luminol, isoluminol, aromatic acridinium esters, imidazoles, acridinium salts, and oxalate esters. Similarly, bioluminescent compounds may be utilized for labelling, the bioluminescent compounds including luciferin, luciferase, and aequorin. Once labeled, the reagent may be employed to identify and quantify ligand (e.g. antibody or antigenic polypeptide) utilizing techniques well-known to the art.

A description of a radioimmunoassay (RIA) may be found in *Laboratory Techniques in Biochemistry and Molecular Biology* [52], with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and

Related Techniques" by Chard, T., incorporated by reference herein. A description of general immunometric assays of various types can be found in the following U.S. Pat. Nos. 4,376,110 (David *et al.*) or 4,098,876 (Piasio).

In a further embodiment, of this invention, commercial test kits suitable for use by a medical specialist may be prepared to determine the presence or absence of predetermined binding activity or predetermined binding activity capability to suspected target cells.

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Further, as used herein, the term "label" refers to a molecule, which may be conjugated or otherwise attached (i.e., covalently or non-covalently) to a binding protein as defined herein. Particularly suitable labels include those, which permit analysis by flow cytometry, e.g., fluorochromes. Preferred fluorochromes include phycoerythrin (P.E., Coulter Corp., Hialeah, FL), phycoerythrin-cyanin dye 5 (PECy5, Coulter), and fluorescein isothiocyanate (FITC, International Biological Supplies, Melbourne, FL). Other suitable detectable labels include those useful in colorimetric enzyme systems, e. g., horseradish peroxidase (HRP) and alkaline phosphatase (AP). Other proximal enzyme systems are known to those of skill in the art, including hexokinase in conjunction with glucose-6-phosphate dehydrogenase. Chemiluminescent labels, such as green fluorescent proteins, blue fluorescent proteins, and variants thereof are known. Also bioluminescence or chemiluminescence can be detected using, respectively, NAD oxidoreductase with luciferase and substrates NADH and FNIN or peroxidase with luminol and substrate peroxide. Other suitable label systems useful in the present invention include radioactive compounds or elements, or immunoelectrodes.

V <u>Diagnostic and therapeutic applications</u>:

This invention provides use of a pharmaceutical composition comprising the reagent as described above, for the prevention or treatment of a autoimmune disease selected from the group consisting of ankylosing

spondylitis, Reiter's disease, psoriatic spondylitis, psoriasis vulgaris and Behcet disease) rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, systemic lupus erythematosus, Sj6gren disease, IDDM, Addison disease, Graves disease, Hashimoto disease, celiac disease, primary biliary cirrhosis, pemphigus vulgaris, epidermolysis bullosa acquisita, Hodgkin's disease, cervical squamous cell carcinoma, multiple sclerosis, optic neuritis, narcolepsy, myasthenia gravis, Goodpasture syndrome and alopecia areata).

In one embodiment the tumor cells are selected e.g. from a group consisting of: melanoma; lymphoma; leukemia; and prostate, colorectal, pancreatic, breast, brain, or gastric carcinoma. Examples of tumors include but are not limited to: sarcomas and carcinomas such as, but not limited to: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's sarcoma, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, germ tumor, non-small cell lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

This invention provides a method for diagnosing a subject with a tumor, comprising the steps of: a) Obtaining a

sample from the subject, b) Contacting the sample with a recombinant reagent as discussed above, wherein the reagent is specific for a specific tumor antigen so as to form a complex, c) Detecting the complex, the presence of which is indicative of a subject having the tumor.

This invention provides a method of detecting a reagent of the recombinant phage library as described above. As provided herein, the junctional regions between the variable domain of the single chain TCR/Ig chimera or single chain TCR contains Cα-TCR or Cβ-TCR N-terminal domain-derived peptides joint to $(Gly_4Ser)_3$. This combined $(Gly_4Ser)_3$ and Cα-TCR or Cβ-TCR N-terminal domain-derived peptides provide novel epitopes for reagents like Ab, which can recognize them with high specificity and affinity, but do not cross react with the TCR molecules on T cells. In addition, Cα-TCR or Cβ-TCR N-terminal domain-derived peptides provide peptide tag at the C-terminal end of the scFv, as a novel epitope for reagents like Ab. However, these reagents are expected to react with with in vivo Cα-TCR or Cβ-TCR and thus with TCR molecules on T cells and can not be used for in vivo imaging.

This invention provides a method for imaging a neoplastic disorder in a subject comprising the steps of administering to the subject an amount of the recombinant reagent as described above, wherein the reagent is labeled, and detecting the label.

For example, reagents such as those described, above may be used to quantitatively or qualitatively determine a subject with a disorder or pathogenic condition. This can

be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled reagent in a subject, and detecting the presense, amount, and/or distrubution but also its localization into the examined tissue.

This invention provides a method of detecting a reagent of the recombinant phage library as described above. This invention provides a method of treating a subject with a disease or a pathogenic conditions, comprising administrating to the subject an effective amount of the reagent as described above, thereby treating the subject with the disease or pathogenic condition.

As used herein, "pharmaceutical composition" means therapeutically effective amounts of the reagent of the invention as described above together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers. A "therapeutically effective amount" as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl., acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts). solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation

enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. In one embodiment the pharmaceutical composition is administered parenterally, intratumorally, paracancerally, transmucosally, transdermally, intramuscularly, intravenously, intradermally, intravascularly, subcutaneously, intraperitonealy, intraventricularly, intracranially.

Further, as used herein "pharmaceutically acceptable carrier" are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobial, antioxidants, collating agents, inert gases and the like.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., *Immunology, Second Ed.*, 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvant include, but are not limited to, complete Freud's adjuvant, incomplete Freud's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol. Preferably, the adjuvant is pharmaceutically acceptable.

Controlled or sustained release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

An active component can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The pharmaceutically acceptable form of the composition includes a pharmaceutically acceptable carrier. In the therapeutic methods and compositions of the invention, a therapeutically effective dosage of the active component is provided. A therapeutically effective dosage can be determined by the ordinary skilled medical worker based on patient characteristics (age, weight, sex, condition, complications, other diseases, etc.), as is well known in the art. Furthermore, as further routine studies are conducted, more specific information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker,

considering the therapeutic context, age and general health of the recipient, is able to ascertain proper dosing. Generally, for intravenous injection or infusion, dosage may be lower than for intraperitoneal, intramuscular, or other route of administration. The dosing schedule may vary, depending on the circulation half-life, and the formulation used. The compositions are administered in a manner compatible with the dosage formulation in the therapeutically effective amount. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous. infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

The present invention provides a kit comprising all the essential materials and reagents required for the library. This generally will comprise selected expression constructs. Such kits will comprise distinct containers for each individual reagent. When the components of the kit are provided in one or more liquid solutions, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being particularly preferred.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXPERIMENTAL DETAILS SECTION

EXAMPLE 1: Construction of Phage display single chain libraries (scTCRVα/TCRVβ, scTCRVα/VL and scTCRVH/TCRVβ).

Set of primers for TCR variable domain amplification: The TCRVα domain is encoded in the human genome by three consecutive gene segments: 5'Vα-Jα-Cα-3'. The gene segments of the TCRVβ domain contain an additional segment, D, that is located between the Vβ and Jβ segments, thus the gene organization is as follows: 5'-Vβ-D-Jβ-Cβ1 or Cβ2.

In order to amplify all known TCR variable regions from a given human mRNA sample, universal oligonucleotides were generated. There are as many as 42 TCRVα and 47 TCRVβ segments and 61 Jα and 13 Jβ gene fragments, and the goal was to amplify as many gene combinations with as few primers as possible.

The oligonucleotides for PCR amplification of the Vα and Vβ gene fragments were designed based on the sequence information published by Arden et al 1995 (1). The 5' end sequences of all described variable TCR gene segments were aligned and grouped by their similarity. Conserved nucleotide sequences were included in the primer sets, and only non conserved ones were exchanged by degenerated sequences as summarized in **Figure 6 and Figure 7** for the Vα and Vβ genes, respectively. In some cases, however, the 5' V-TCR sequence could not be grouped and the oligonucleotides were designed only for a specific TCR V gene segment. In addition, the primers included at their 5'end NcoI or SalI restriction sites for cloning. To facilitate the PCR reactions, the 3' primers were designed to anneal to the 5' end of Cα or Cβ gene segments. As a result, the amplified TCRVα segments contained a stretch encoding seven amino acids from the Cα segment, and the amplified TCRVβ segments contained a stretch encoding six amino acids at the 3'-end, originated from the Cβ1 or Cβ2 segments. Both Cα and Cβ primers include XhoI and NotI restriction sites, respectively, which are needed for cloning.

Amplification of TCR gene segments: The total RNA used for TCR gene amplification was obtained from $1.2x10^9$ pooled white blood cells, freshly collected. RNA and messenger RNA (mRNA) were prepared according to standard protocols using Promega RNAgents Total RNA Isolation System and PolyATtract mRNA Isolation System (Promega, Madison, USA). The quality of total RNA prepared was then eval :ed by formaldehyde gels according to standard procedures (Maniatis et al., 2).

RT-PCR was prepared by employing the Access RT-PCR system and the Access RT-PCR Introductory system from Promega following the manufacturer's instructions (Promega, Madison, USA). The Access PCR system includes an optimized single-buffer system that permits extremely sensitive detection of RNA transcripts, without a requirement for buffer additions between the reverse transcription and PCR amplification steps. Thus, combination of TCR-specific oligonucleotides for Vα or Vβ, together with their respective primers Cα or Cβ1 or Cβ2 were used for each RT-PCR reaction. The PCR reaction was done as in Nissim et al (3). Briefly, 1µl of DNA from the RT-PCR reaction was individually amplified using amplified using Vα and Cα or Vβ and Cβ1 or Cβ2 primers in a volume of 50µl with 250µM dedeoxynucleotide triphosphates (dNTPs), 10mM KCl, 10mM $(NH_4)_2SO_4$, 20mM TrisHCl pH8.8, 2mM $MgCl_2$, 100µg/ml bovine serum albumin (BSA) and 1µl (1 unit) of Taq DNA polymerase. 30 cycles (94°C-2 min, 55°C-1 min and 72°C-2 min) were used.

Preparation of Immunoglobulin (Ig) VH and VL segments: As it is the first time that phage display chimeric Ig/TCR is constructed, it was necessary to use known feature of several Ig variable domain proteins as a marker to test the integritiy of this novel construct; for example, the fact that VH3 familiy bind to protein A (4) and DPK12 to protein L (5). Thus we first tested our construct by fusing our TCR gene fragments to either VH3 or DPK12. The source of VH3 could originate from VH3 amplified PCR originated from the Nissim library, or from individual selected clones using this library bearing VH3 gene segments (e.g. anti-NIP or anti-phOx clones bearing DP-47 VH gene segment). Alternatively the commercially available antibody phage display libraries

"Tomlinson A" and "Tomlinson B" (MRC, Cambridge UK, unpublished) were employed. These libraries contain the DP47 gene segment belonging to the VH3 heavy chain gene family (4). As a source for VL we had two alternatives: either DPK12 gene from "Tomlinson A" and "Tomlinson B" or DPK12 from several other libraries available (Nissim et al, unpublished). The expression of the TCR/Ig chimera could then be evaluated by an ELISA using horseradish peroxidase (HRP)-coupled protein A and HRP-conjugated protein L.

To obtain the VH and VL inserts, PCR reaction using V-family based oligonucleotides were imployed as described (Marke et al, 6; Coks et al, 7; Williams et al, 8). If "Tomlison" library were used pTI plasmid vectors containing a sample of the library were prepared by QIAGEN plasmid purification kit according to manufacturer's instructions (QIAGEN, Germany). Purified plasmids or PCR products were then digested with the restriction enzymes NcoI/XhoI to obtain the VH segment and SalI/NotI (all from Boehringer, Mannheim, Germany) to obtain the VL gene segment (see Fig. 1). After overnight digestion, the V segments were gel purified according to manufacturer's instructions using the QIAGEN DNA purification kit. The sizes of the V gene segments obtained from these digests were about 350 bp.

Currently, to construct the chimeric TCR/Ig library, the Ig V gene segments are amplified from the same RNA pool used for the amplification of the TCR gene segments. RT-PCR is performed using VH and VL family-based primers in combination with their respective JH, JK and Jλ primers. Depending on the vector used to consytruct the libraries, the VL oligonucleotides were used as published or diversified to exchange ApaLI with SalI (Marke et al, 6; Coks et al, 7; Williams et al, 8). RT-PCR and PCR are performed as described above in case of the TCR V genes (section Ba).

Vectors: The following vectors were used: phage vectors, that encode the pIII-fusion protein and all functions required for replication, packaging and infection of bacteria, and phagemid vectors which require "rescue" with a helper phage. Phagemid vectors comprise the pIII-fusion, plasmid and phage origins of replication and antibiotic resistance markers; the helper phage provides the functions necessary for single strand DNA replication and

packaging. Helper phages are poorly packaged in comparison with agemids due to a defective M13 origin of replication (Vieira and Messing, 9).

The basic vector for the library construction is pHEN1 phagemid vector (REF). In pHEN1, the production of the pIII-fusion protein is under the control of the lacZ promotor which is inhibited with glucose and induced with isopropyl-D-thiogalactoside (IPTG) (De Bellis and Schwartz, 10). pHEN1 can also be used directly for expression of the recombinant antibodies as soluble fragments since an amber stop codon is located at the junction of the antibody gene and gIII. When grown in suppressor strains of *E. coli* such as TG1 (Gibson, 11), the gIII-fusion protein is produced and packaged into phage, while growth in non-suppressor strains such as HB2151 (Carter et al., 12) allows the secretion of soluble antibody fragments into the bacterial periplasm, and into the culture broth.

To check the feasibility of our concept it was convenient to us to use one of the pHEN1 modified vector named pTI (kindly donated by I. Tomlinson, MRC, Cambridge, UK) or pAN (Nissim et al, unpublished). As seen in Fig. 2, the phagmid vector is the same vector as the pHEN1 vector which includes NcoI and XhoI restriction sites for cloning the VH/L or TCR$\alpha/\beta/\gamma/\delta$ gene segments at the 5' end. However, it include additional SalI or ApaLI and NotI sites for cloning VH/L or TCR$\alpha/\beta/\gamma/\delta$ at the 3' end. Both pTI and pAN vectors, provide a myc tag (13) and a His tag (14) flanking the NotI site for detection and purification, respectively, and (Gly4Ser)3 linker between the V segments.

Cloning of TCR and Ig variable gene segments: For the first generation of constructs we were focused on TCR V genes in combination with VH3 and DPK12. After checking that for each of the PCR-amplified samples a band of the appropriate size was obtained on agarose gel electrophoresis, the PCR products of each of the amplifications encoding the different V segments were pooled as follows: pool (a) TCR-Vα ; pool (b) TCR-Vβ1; pool (c) TCR-Vβ2; pool (d) V_H-DP47; pool (e) VL-DPK12. The individual pools were purified by a PCR purification kit (Boehringer, Mannheim, Germany). After over night digestions with NcoI and XhoI restriction enzymes for Vα or VH, and SalI and

NotI restriction enzymes for Vβ1, Vβ2, or VL, respectively, fragments were gel purified and cloned into separate phagmide vectors (Fig2):

1. scTCRVα/VL: Vα between NcoI/XhoI and VL between SalI/NotI restriction sites;

2. ScTCRVH/Vβ: VH between NcoI/XhoI and Vβ between SalI/NotI restriction sites; 3. scTCRVαVβ: Vα between NcoI/XhoI and Vβ between SalI/NotI restriction sites

After ligation of 1µg of each plasmids and inserts, *E.coli* TG-1 bacteria were electroporated (15). The diversity of about 10^8 different clones were obtained for each scTCRVα/VL, or scTCRVH/Vβ, or scTCRVα/TCRVβ. Trasformed bacteria were scraped from plate and kept in 15% glycerol in 2XTY at –70°C. Thereafter, 50µl bacteria of each library were used to rescue phage particles with helper phage VCS-M13 (Stratagene).

As previously described (Marks et al., 1991, 16; Hoogenboom and Winter, 1992, 17; Griffiths et al., 1993, 18), phage were rescued from the entire library or from single ampicillin-resistent colonies of infected TG-1 cells (K12, Δ(lac-pro), supE, thi, hsdD5/F'traD36, proA+B+, laclq, lacZDM15) using helper phage VCS-M13 (Stratagene). Soluble fragments were induced from single colonies of infected HB2151 bacteria ((K12, ara, Δ(lac-pro), thi/F'proA+B+, laclqZDM15) (Hoogenboom et al., 1991, 19)) by IPTG. Bacterial supernatants containing phage or sclg/TCR or scTCR fragments were screened for their s / phage titration PCR, ELISA and SDS gel electophoresis (take ref from Mark).

*Phage titration: P*hage were rescued from the entire library or from single ampicillin-resistant colonies of infected suppressor TG-1 bacteria using the helper phage VCS-M13 as described above. Bacterial supernatants containing phage fragments were used to re-infect new TG-1 bacteria that

were grown to the log phase ((OD at 600 nm of 0.4-0.5 (Phage/phagemid infect F+-E. coli via the sex pili. For sex pili production and efficient infection *E. coli* must be grown at 37^{0}C and be in log phase)). Finally, the titer of the phage particles of the various libraries as well as that of individual colonies was evaluated.

PCR and Sequencing: In order to assess the completeness of the integration of the individual TCR chains of scTCRVα/VL, scTCRVH/Vβ, and scTCRVαVβ constructs, single ampicillin-resistant colonies of infected *E. coli* TG-1 were used as template for PCR screening using the following primer combinations (Fig 3):

LMB3 (5'-CAGGAAACAGCTATGAC) and fdSEQ (5'-GAATTTTCTGTATGAGG), to measure the size of the entire insert which is expected to be about 900bp;

LMB3 and Cα–For-XhoI (Fig.6) to prime the Vα segment (500bp fragment only when priming the scTCRVα/VL or the scTCRVαVβ constructs);

LMB3 and Cβ–For-NotI (Fig.7) to prime the Vβ segments (900bp fragment only when priming the scVH/TCRVβ or scTCRVαVβ constructs).

Different clones were sequenced by the dideoxy method (Sanger et al., 1977, 20) using DyeDeoxy chain termination (Applied Biosystems Inc.) and an Applied Biosystems 373A DNA sequencer. The sequences were analysed by SeqEd (Applied Biosystems Inc.) and MacVector 3.5 (IBI Kodak, New Haven, CT).

Phage ELISA: ELISA plates were coated with phage supernatant for two hours at 37°C. Plates were then washed three times with Phosphate Buffered Saline (PBS), and 2% marvel in PBS was added for additional two hours of incubation in order to block the free plastic surface. Plates were re-washed three times with PBS-0.05% Tween 20, followed by 3 washes with PBS alone and horseradish peroxidase (HRP)-conjugated - anti-M13 polyclonal antibodies were added for an additional hour. After a further washing step, the

ELISA was developed with 100µl substrate solution (100 µg/ml 3,3',5,5'-tetra methylbenzidine (TMB) in 100 mM sodium acetate, pH 6.0, and 10 µl of 30% hydrogen peroxide per 50 ml of this solution directly before use). The reaction was stopped by adding 50 µl of 1 M sulphuric acid to each well. Plates were read at OD 450 and OD650; the final readout was the difference between the two values (OD650 – OD450). To detect the display of scTCRVα/VL and scTCRVH/Vβ on the surface of the phage particles, HRP-conjugated protein L and HRP-conjugated protein A were employed: (i)scTCRVα/VL should interact with Protein L but not protein A, (ii) scTCRVH/Vβ should interact with Protein A but not protein L, and (iii) scTCRVαVβ should not interact neither with Protein L nor with protein A, and (iv) sclgDP47-DPK12 should react with both reagents.

Expression of Ig-TCR and TCR fragments : Soluble fragments were induced from single colonies of infected non-suppressor HB2151 bacteria (Hoogenboom et al., 1991, 19) by IPTG. Briefly, HB2151 were infected with phage produced from individual TG-1 clones which had been screened as described above. Individual colonies were picked into 100 µl 2xTY-AMP-GLU in 96-well plates, grown overnight, transferred in a small inoculum (about 2 µl or using a 96-prong transfer device) to a second 96-well plate containing 200 µl 2xTY-AMP-0.1% GLU and grown to OD 0.9, IPTG to give a final concentration of 1 mM IPTG was then added and incubation was continued for further 16 to 24 hr by shaking at 30°C. Plates were then centrifuged at 1,800xg for 10 min and 100 µl of the supernatant was used for ELISA. In addition, bacterial periplasm (as described below) was prepared and tested in both ELISA and SDS gels.

Harvesting Fragments from the Periplasm: Induced bacterial cultures were centrifuged at 10,800xg for 15 min, resuspended in 1/20 the original volume with 30 mM Tris, pH 7.0-20% sucrose-1 mM EDTA and left for 20 min on ice. Tubes were centrifuged at 10,800xg for 15 min and supernatants collected (periplasmic fraction) to a new tube. Pellets were resuspended in 1/20 the original volume with 5 mM $MgSO_4$ and incubated further for 20 min on ice.

After centrifugation at 10,800xg for 15 min, supernatant (osmotic shock fraction) was combined with the periplasmic fraction.

Western blotting: Phage was purified using polyethylene glycol (PEG) precipitation of supernatants collected from infected bacteria by adding 1/5 volume PEG/NaCl (20% PEG 6000-2.5 M NaCl) and incubation for 1 hr at 4°C. Tubes were centrifuged at 10,800xg for 30 min. and pellets were resuspended in 1/10 of the original volume in water. Western blots of M-13 gIII and scFv-gIII or scIg/TCR-gIII or scTCR-gIII fusion proteins were conducted as follows: 20µl of each purified phage or periplasmic preparation were separated on a 10% SDS–acrylamide gel and then electroblotted. The nitrocellulose membran (Schleicher&Schuell, Dassel, Germany) was blocked overnight at 4°C in 10% marvel in PBS/0.5% Tween 20. After washing with PBS/0.5% Tween 20, gIII and scFv-gIII or scIg-TCR-gIII or scTCR-gIII fusion proteins were detected with HRP-conjugated anti-M13 gIII conjugate, or protein A-HRP or protein L-HRP. Peroxidase activity was detected using the ECL kit from Amersham Amersham Life Science, Little Chalfont, UK).

Target: Selection is performed against any target which include purified proteins, peptides, cells, body fluid, organ etc.

Selection of recombinant scTCR and scTCR-Ig display: Phage display libraries was enriched for antigen-binding scTCR and scTCR-Ig clones by subjecting the phages to repetitive rounds of selection including binding, washing and elution steps, re-infection into bacteria and growth to re-express the scTCR or scTCR-Ig molecules on the phage surface. A combination of procedures which have already been shown to work for the selection of scFv antibody fragments was applied (9, 10). Briefly, to select for phage particle(s) expressing scTCR or scTCR, immunotubes (Nunc-Immuno Tubes, MaxiSorp, Nunc, Denmark) was coated with the target protein by adding 10-100µg/ml of protein in PBS, and incubated overnight at 4^0. The uncoated sites on the immunotubes were then be blocked by 2% skimmed milk in PBS for two hours. After washing with PBS tubes will be exposed to 10^{12}-10^{13} transforming units (t.u.) of the phage library in 2% skimmed milk in PBS. After two hours incubation the library was washed free of unbound phage and the bound

phage will be eluted by alkali (100 mM triethylamine) and neutralized with 1M Tris, pH7.4. For enrichment of the target bound phage, the eluate was used to infect exponential E.coli TG-1 and after growing them over night, phage particles was rescued with VCS-M13 helper phage (Stratagene, USA) as described (9, 10). The process was repeated two to five times and final phage preparation was used to infect TG-1 bacteria to screen for individuals TG-1 colonies bearing specific phage.

To take the selection technology a step further towards the situation of interactions in vivo, the selection will be performed in solution by binding to biotinylated target, followed by capture of bound phage with streptavidin-coated paramagnetic beads. Selection will be performed as in case of immunotubes however, if biotin disulphide is used to link the antigen to biotin, the phage antigen complex can be eluted by use of dithiothreitol (DTT) which disrupts the disulphide linkage between biotin and antigen.

Additional step will include cell selection, since the ultimate goal must be the production of reagents, which are able to detect their targets in native state on tumor cell surfaces. In case of HLA/peptide target peptide transporter-deficient cell lines like BM36.1 (HLA-A1,Cw4,B35,Bw6) (11) or T2 (HLA-A2) will be employed. These cells can be grown for one day at 26°C and then loaded with the desired peptide (e.g. a MAGE-1-derived, HLA-A1-binding peptide). Moreover, counterselection involving cell selection technology in combination with selection against purified complexes will be employed. For example, selection for scTCR or scTCR-Ig molecules against HLA-A1/MAGE-1 peptide complexes will first involve two consecutive steps in which the MAGE peptide is represented in the context of two different HLA molecules, such as HLA-A2 and HLA-B44 molecules. This counterselection will eliminate those phage particles reacting with "public" determinants on HLA class I. Further selections will then be carried out using a mixture of HLA-A1/MAGE1 complexes as targets or HLA-A1 typed BM36.1 cells loaded with MAGE1 peptide. Variations on this theme are obviously possible and will be carried out.

Screening assays and specificity/affinity measurement of scTCR and scTCR-Ig molecules: The progress of selection can be readily monitored after each round by two simple tests. Firstly, the number of infective phage (transforming units; t.u.) eluted after each round provides a first hint of progress. Typically,the numbers are low in the early rounds (about $10^5/10^6$ t.u.), increasing in later rounds to about 109 t.u. Secondly, the binding of the phage population (or individual clones) can be detected by ELISA, detecting the bound phage with anti-phage enzyme-linked antisera (eg. anti-M13 horseradish peroxidase (HRP)). Depending on the power of selection, binding is usually detected after two to five rounds of selection. The selection is usually terminated when most of the phage clones are found to bind by ELISA. At this stage soluble fragments will be expressed and secreted directly using HB2151 non-suppressor strain. The expression of antibody fragments is driven from lacz promoter, and is therefore induced by IPTG. The myc peptide tag appended to the antibody fragment facilitate their detection in ELISA by anti-myc tag antibodies (Sigma). ELISA will be done with various antigens coated onto the wells of microtiter plates or being presented on fixed cells. However, since the ultimate goal must be the production of reagents which are able to detect their targets in native state on tumor cell surfaces, flow cytometric analysis of BM36.1 loaded with the target peptide (e.g. a MAGE-1-derived, HLA-A1-binding peptide) will be performed. The affinity of the binders for their antigens can be measured by Fluorescence Quenching (12), Competition ELISA (13), or most accurately, by Surface Plasmon Resonance (BIACore) (14).

The specificity of the recombinant scTCRs or scTCR-Ig to be produced will then be rigorously assessed by screening against a large panel of HLA-typed cell lines, their capacity to inhibit the lytic activity of CTL clones specific for various antigens, their capacity to inhibit the production of TNF or IFNg by stimulated CTL, and immunoprecipitation and Western blotting of tissue extracts.

Assessment of the diagnostic potential of recombinant scTCR molecules: Recombinant scTCR or scTCR-Ig molecules showing specificity for a defined

HLA/peptide combination are expected to be extremely versatile tools in tumor diagnostics. So far, the presence of a given antigen on the surface of tumor cells is deduced from the HLA type of the patient and from RT-PCR analysis of the expression of the relevant gene in the sample. It is obvious, though, that the expression of the gene does not necessarily mean that the antigenic peptide will be present in sufficient amount at the surface of the cells. This problem is becoming more important now that pilot vaccination studies have started with cancer patients selected on the basis of the criteria mentioned above. It is quite possible that some patients are included in these studies although the actual expression of the antigen at the surface of the tumor cells in vivo is extremely low and consequently irrelevant. In tissue sections, suitable recombinant scTCRs and scTCR-Ig would allow to define precisely the proportion and distribution of antigenic tumor cells within a solid tumor, an information relevant to anti-tumor vaccine development and as yet impossible to obtain. Furthermore, such recombinant reagents would react with their target not only when it is expressed on the tumor cell surface, but presumably also when it is shed into body fluids.

The latter may allow monitoring of tumor progression, remission or relapse,comparable to the detection of the products of certain HLA class I alleles which are shed by cells from transplanted organs during rejection episodes.

Assessment of the therapeutic potential of selected recombinant scTCR and scTCR-Ig molecules in SCID mice: Selected recombinant scTCR or scTCR-Ig molecules will be engineered into fusion proteins with a truncated form of Pseudomonas exotoxin (PE38) (15), which will be expressed in E. coli followed by isolation from intracellular inclusion bodies by refolding and purification. The specificity and sensitivity of the immunotoxin fusion-proteins will be tested using several cellular model systems, in vitro and in vivo. Immunodeficient SCID mice transplanted with three different human tumors (~5mm diameter) will be employed to assess the functional activity of scTCR-PE38 or scTCR-Ig-PE38 constructs in vivo in comparison with other therapeutic agents such as CTL with specificity for HLA/peptide complexes.

RESULTS

Construction of Libraries: Amplification of the TCR Vα and Vβ gene segments was done using specifically designed TCRV gene-based primers and RNA prepared from lymphocytes .

The subgroup-specific TCR primers gave single RT-PCR bands of varying intensities. After PCR re-amplification of each RT-PCR reaction, the various TCR Vα, Vβ, VH and VL were cloned into the phagmid vector, building three different libraries: (i) TCRVα/TCRVβ, (ii) chimaeric TCRVα/VL and finally (iii) chimaeric TCRVH/Vβ (Fig1). The diversity of each of these libraries was between 10^7 to 10^8 different clones, as estimated by counting colonies plated after electroporation. From each library, several samples were sequenced and analyzed using the TCR nucleotide database. This database contains human sequences of 87 Vα regions, 148 Vβ regions, 61 Jα regions, 17 Jβ regions and 2 Dβ. Sequences for Vα and Vβ were retrieved from the database that was submitted to the EMBL nucleotide sequence database by Arden et al. (1) and assigned the alignment number DS23485. The sequences for the Jα regions, Jβ regions and the two Dβ were retrieved from the IMGT database. Alignment for the cloned sequences was carried out against this local database using MacVector 6.1 (Oxford Molecular Ltd. Sequence Analysis Software for Macintosh).

Sequenced samples from the libraries generated as described above have shown a random representation of different TCRVα and TCRVβ gene segments. In addition, the various Jα and Jβ segments were also represented in the library (Fig8).

Evaluation of constructed libraries: Libraries were evaluated for phage display and soluble scTCR/Ig production by: phage titer, sequence analysis, ELISA, SDS gel electrophoresis and Western blotting.

Phage Display: Several TG-1 clones harbouring the pTI vector which includes either TCRV$_\alpha$/V$_L$ or TCRVH/V$_\beta$ and TCRVα/ TCRVβ were studied.

Phage titers of the various libraries: The titers of theTCRVα/VL, TCRVH/Vβ, chimaeric TCRVα/ TCRV$_\beta$ libraries were in the range of 10^9 for each of the library, and for individual clones originating from them as well. This result must be taken as an indication that the TCR and the chimaeric fusion proteins are not toxic and that phage with these fusion proteins can be propagated.

Screening for relevant inserts: Individual clones from chimaeric TCRVα/VL and TCRVH/V$_\beta$ were first screened by PCR for their relevant inserts using the following primers: LMB3 and Cα-For-XhoI for the TCRVα/V$_L$ library (Fig.3, 6) and LMB3 together with C$_\beta$-For-NotI for TCRVH/Vβ library (Fig.3, 7). Positive PCR clones were re-amplified by LMB3 and fdSEQ for sequence analysis and the presence of random combinations of various TCRV$_\alpha$-J$_\alpha$ and TCRV$_\beta$-J$_\beta$ genes was demonstrated (Fig8, 9).

Analysis of TCR and Ig-TCR phage expression: In order to estimate the functionality of the TCR and the chimaeric scIg/TCR proteins displayed on phage, ELISA was performed using phage supernatant. Bound phage were detected with anti-M13-HRP, Protein A-HRP and Protein L-HRP. However, it was important to use the same phage supernatant for the parallel analysis of TCR and Ig V domain proteins existing in the newly constructed libraries. Phage originating from the TCRVα/VL library reacted with anti-M13 and Protein L-HRP, but not with Protein A-HRP (Fig 3A). Phage originating from the TCRVH/Vβ library reacted with anti-M13 and Protein A-HRP, but not with Protein L-HRP (Fig 3B). These results demonstrate the integrity of the various chimaeric domain combinations at the level of phage display. Soluble TCRVα/TCRVβ or phage displayed TCR did not react with neither Protein A or L. Antibody reagent specifically against the new linker and tag is currently prepared which will be applied in the future ELISA.

SDS gel analysis and Western blotting: SDS gel analysis confirmed that the size of gIII and gIII-sc fusion were 60kD and 100kD, respectively, as expected. The same results were obtained by immunoblotting using anti-M13-HRP or Protein L-HRP (in case of TCRVα/VL) or Protein A-HRP (in case of TCRVH/Vβ) that detected mainly bands of 100kD. Similar results were obtained when purified phage originating from the library pool or from individual clones were analyzed.

Analysis of soluble scTCR and sclg-TCR : Soluble (protein fragments detached from phage) scTCRV$_\alpha$/TCR V$_\beta$, and and chimaeric sclg/TCR expression analysis was performed exactly as for the evaluation of the constructed libraries (see above). However, anti-M13 antibody was omitted. HB2151 clones harbouring the pTI vector which included either TCRV$_\alpha$/TCRV$_\beta$, chimaeric TCRVα/VL and TCRVH/Vβ were first screened by PCR for the presence of their relevant inserts using the same primer combinations as in (1). Only the positive PCR clones were taken for further sequence analysis. Periplasmic extracts originating from the same clones were used in parallel for ELISA with Protein A-HRP and Protein L-HRP, and SDS-PAGE analysis. As expected, scTCRVα/VL reacted with Protein L-HRP and scTCRVH/Vβ with Protein A-HRP in ELISA (Fig.3C). A major band of 30kD which corresponds to the scTCR/Ig and TCRV$_\alpha$/TCRV$_\beta$, fragment was detected in SDS-PAGE.

Linkers: TCRV gene amplification was done by a set gene-based primers annealing to the 5' end of TCRV genes together with a 3' primer, which hybridises to the 5' end of Cα or Cβ, resulting in RT-PCR amplification of each of the TCRV gene segments with a 3' tail originating from the constant region of the TCR genes. These tails are maintained in the library at two positions: (i) linker between Vα and Vβ and (ii) as additional 3' tag (Figure 5) As demonstrated above, these new linkers did not interfere with the expression of the intact scTCR molecules (Fig 4). This is not the first time that a non-Gly4Ser linker is used. However, usually they are employed in commercially available antibodies or linkers, facilitating recombination. By this

approach, not only the number of PCR reactions for the TCRV gene amplification has been reduced by two logs, but for the first time specific linkers and a tag have been developed which can be specifically used to detect our novel constructs.

Modification of TCR Libraries Based on the Structure of the TCR: These results indicate that the difficulties in obtaining expression of intact and soluble scTCR as well as chimaeric constructs could be overcome. Still, additional improvements could be included in further constructed libraries. For example, mutations in scTCR genes which incorporate stabilizing motives based on the structural analysis of both TCR and antibodies can be included. There are two regions within the TCR molecule that drew our attention based on the work of Kieke et al. (21), in which a mutated scTCR was expressed in yeast . One region refers to the "elbow-like" structure in Cβ chain that stabilizes the scTCR. VβA13 and VβG17 (Figure 5) belong to the FR1 region. In a TCR molecule, this region is positioned in proximity (7-10Å) to a negatively charged "elbow" generated by Glu221, Glu 222 and Asp 223 of the $C_\beta$domain (Figure 6, white). This charged "elbow" does not exist in antibodies and is unique to TCR molecules. It is stabilized by the high positive charge that characterizes the Vβ FR1 of the TCR. (The net charge of residues 8-17 of Vβ is +1.6, as calculated from Vβ sequences from the Kabat database). The positive charge of the Vβ FR1 is stabilized as a result of electrostatic interactions with the negatively charged "elbow" of TCR Cβ. In the absence of this "elbow", as in scTCR, the presence of negatively charged amino acids and more hydrophobic buried residues, as achieved by VβG17E and VβA13V mutations, respectively, stabilizes the positively charged region of Vβ FR1 and thus stabilizes scTCR folding. This assumption is confirmed by the fact that the FR1 sequence in this region in TCRVα, Ab-VH, Ab-Vκ and Ab-Vλ chains have a significantly lower positive charge (mean net charge of +0.4), and that scFv can be displayed without problem.

The second refers to the interface between Vα and Vβ which might be a target for further development: VαL43, VαL104 and VβT105 are involved in the

Vα/Vβ interface. VαL43 belongs to the Vα FR2. The VαL43P mutation generates a leucine-proline pair at the Vα/Vβ interface (instead of leucine-leucin pair). This pair is highly conserved in antibodies and stabilizes the Vα/Vβ interface and increases stability of the mutant, facilitating its secretion and display on yeast cells (Kieke et al., 21). It is interesting to note that VβA13V,VβG17E and VαL43P mutations are naturally found in many VH and VL sequences (21). In addition, VαL104 and VβT105 belong to the CDR3 loop. It should be noted that the VαL104P mutation reduces the affinity of the scTCR, probably since proline introduces conformational changes in the V_{α}CDR3 that is involved in the binding of the target. These mutations as described above can be included in construction of TCR libraries.

EXAMPLE 2 **To test the performance of the newly constructed library selection were performed against several targets as described in the methods section: recombinant IgE receptor (IgER), Epidermal growth factor (EGF), a** human epidermoid carcinoma cells (A431) which over express a very high number of EGF receptors, FITC-BSA,and HLA-A2/MAG3 and HLA-A2/MAGE10 complex. All selections have been performing very well, and specific clones were selected. Fig10 shows sequence sampling of some of these selections.

What is claimed is:

1. A phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent from each of the recombinant phages.

2. The library of claim 1, wherein the TCR recognition element comprises a variable fragment of the TCR.

3. The library of claim 2, wherein the variable fragment of the TCR comprises one or more of TCR variable α(TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains.

4. The library of claim 3, wherein the TCRVα comprises one or more of the complementarity determining residues (CDR) 1, CDR2 or CDR3 segments.

5. The library of claim 3, wherein the TCRVβ comprises one or more of the CDR1, CDR2 or CDR3 segments.

6. The library of claim 3, wherein the TCRVγ comprises one or more of the CDR1, CDR2 or CDR3 segments.

7. The library of claim 3, wherein the TCRVδ comprises one or more of the CDR1, CDR2 or CDR3 segments.

8. The library of claim 1, wherein the TCR recognition element comprises a constant fragment.

9. The library of claim 8, wherein the constant fragment of the TCR is Cα, Cβ1, Cβ2, Cγ or Cδ domain.

10. The library of claim 1, wherein the immunoglobulin recognition element is an antibody comprising a variable domain.

11. The library of claim 10, wherein the antibody comprises a heavy chain and/or a light chain

12. The library of claim 11, wherein the antibody comprises a heavy chain .

13. The library of claim 10, wherein the antibody comprises a light chain.

14. The library of claim 12, wherein the heavy chain comprises one or more heavy chain variable fragments (VH).

15. The library of claim 13, wherein the antibody comprises one or more light chain variable fragments (VL).

16. The library of claim 12, wherein the heavy chain comprises one or more C_H1 constant domains.

17. The library of claim 13, wherein the light chain comprises one or more Cκ (kappa) or Cδlambda) domains.

18. The library of claims 14 or 15, wherein the variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.

19. The library of claim 1, wherein the reagent is a Fv fragment.

20. The library of claim 19, wherein the Fv fragment is a single chain Fv fragment.

21. The library of claim 1, wherein the reagent is a Fab ~~Fv~~ fragment.

22. A linker which joins the T-cell receptor (TCR) recognition element, and the immunoglobulin (Ig) recognition element of the reagent comprising a nucleic acid which is characterized as: i) aiding in folding of the domains, ii) supporting the stabilization of the intact protein construct.

23. The linker of claim 22, wherein the nucleic acid comprises the sequence as set forth in Figure 5 .

24. The library of claim 1, wherein the vector comprises a nucleic acid which codes for a second molecule that is linked to the TCR and/or the chimeric TCR/Ig reagent.

25. The library of claim 24, wherein the second molecule is a molecule which interacts with a second,

nonoverlapping determinant of the target molecule or a multimeric target .

26. The library of claim 25, wherein with the second molecule enhances the overall avidity of the interaction of the TCR and/or the chimeric TCR/Ig fragment with the target molecule or a multimeric target.

27. The library of claim 26, wherein the TCR and/or the chimeric TCR/Ig fragment joint to the second molecule is a bispecific molecule.

28. The library of claim 26, wherein the second molecule is a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof.

29. The library of claim 28, wherein the second molecule is is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant thereof which exhibits low affinity to their respective target.

30. A tag which joins the T-cell receptor (TCR) reagent, and the chimeric reagent with gIII protein of the bacteriophage of the reagent comprising a nucleic acid which is characterized as: i) aiding in purification and detection of the reagent.

31. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVα/VL and/or mutation and variant thereof.

32. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVβ/VL and/or mutation and variant thereof.

33. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVα/VH and/or mutation and variant thereof.

34. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVβ/VH and/or mutation and variant thereof.

35. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VL/TCRVα and/or mutation and variant thereof.

36. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VL/TCRVβ and/or mutation and variant thereof.

37. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VH/TCRVα and/or mutation and variant thereof.

38. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VH/TCRVβ and/or mutation and variant thereof.

39. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVγ/VL and/or mutation and variant thereof.

40. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVδ/VL and/or mutation and variant thereof.

41. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVγ/VH and/or mutation and variant thereof.

42. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVδ/VH and/or mutation and variant thereof.

43. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VL/TCRVγ and/or mutation and variant thereof.

44. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VL/TCRVδ and/or mutation and variant thereof.

45. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VH/TCRVγ and/or mutation and variant thereof.

46. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VH/TCRVδ and/or mutation and variant thereof.

47. A phage-display library for screening for target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant

thereof; and in which the vector expresses a recombinant TCR recognition element from each of the recombinant phages.

48. The library of claim 47, wherein the TCR recognition element comprises a variable fragment of the TCR.

49. The library of claim 48, wherein the variable fragment of the TCR comprises one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains.

50. The library of claim 49, wherein the TCRVα comprises one or more of the complementarity determining residues (CDR) 1, CDR2 or CDR3 segments.

51. The library of claim 49, wherein the TCRVβ comprises one or more of the CDR1, CDR2 or CDR3 segments.

52. The library of claim 49, wherein the TCRVγ comprises one or more of the CDR1, CDR2 or CDR3 segments.

53. The library of claim 49, wherein the TCRVδ comprises one or more of the CDR1, CDR2 or CDR3 segments.

54. The library of claim 47, wherein the TCR recognition element comprises a constant fragment.

55. The library of claim 54, wherein the constant fragment of the TCR is Cα, Cβ1, Cβ2, Cγ or Cδ domain.

56. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVα/TCRVβ and/or mutation and variant thereof.

57. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVα/TCRVγ and/or mutation and variant thereof.

58. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVα/TCRVδ and/or mutation and variant thereof.

59. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVβ/TCRVα and/or mutation and variant thereof.

60. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVβ/TCRVγ and/or mutation and variant thereof.

61. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVβ/TCRVδ and/or mutation and variant thereof.

62. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVγ/TCRVα and/or mutation and variant thereof.

63. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVγ/TCRVβ and/or mutation and variant thereof.

64. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVγ/TCRVδ and/or mutation and variant thereof.

65. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVδ/TCRVα and/or mutation and variant thereof.

66. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVδ/TCRVβ and/or mutation and variant thereof.

67. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVδ/TCRVγ and/or mutation and variant thereof.

68. The library of claim 47, wherein the vector comprises a nucleic acid which codes for a second molecule that is linked to the reagent

69. The library of claim 68, wherein the second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target .

70. The library of claim 69, wherein the second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target.

71. The library of claim 68, wherein the TCR fragment joint to the second molecule is a bispecific molecule.

72. The library of claim 68, wherein the second molecule is a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof.

73. A phage displayed recombinant chimeric TCR recognition element/Ig recognition element reagent.

74. A soluble reagent detached from phage which includes recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent.

75. The reagent of claim 74, wherein the TCR recognition element comprises a variable fragment of the TCR.

76. The reagent of claim 75, wherein the variable fragment of the TCR comprises one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains.

77. The reagent of claim 76, wherein the TCRVα comprises one or more of the complementarity determining residues (CDR) 1, CDR2 or CDR3 segments.

78. The reagent of claim 76, wherein the TCRVβ comprises one or more of the CDR1, CDR2 or CDR3 segments.

79. The reagent of claim 76, wherein the TCRVγ comprises one or more of the CDR1, CDR2 or CDR3 segments.

80. The reagent of claim 76, wherein the TCRVδ comprises one or more of the CDR1, CDR2 or CDR3 segments.

81. The reagent of claim 74, wherein the TCR recognition element comprises a constant fragment.

82. The reagent of claim 81, wherein the constant fragment of the TCR is Cα, Cβ1, Cβ2, Cγ or Cδ domain.

83. The reagent of claim 74, wherein the immunoglobulin recognition element is an antibody comprising a variable domain.

84. The reagent of claim 83, wherein the antibody comprises a heavy chain and/or a light chain

85. The reagent of claim 84, wherein the antibody comprises a heavy chain.

86. The reagent of claim 84, wherein the antibody comprises a light chain.

87. The reagent of claim 84, wherein the heavy chain comprises one or more heavy chain variable fragments (VH).

88. The reagent of claim 84, wherein the antibody comprises one or more light chain variable fragments (VL).

89. The reagent of claim 84, wherein the heavy chain comprises one or more C_H1 constant domains.

90. The reagent of claim 84, wherein the light chain comprises one or more Cκ (kappa) or Cδ (lambda) domains.

91. The reagent of claim 87, wherein the variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.

92. The reagent of claim 83, wherein the reagent is a Fv fragment.

93. The reagent of claim 92, wherein the Fv fragment is a single chain Fv fragment.

94. The reagent of claim 83, wherein the reagent is a Fab fragment.

95. The reagent of claim 94, wherein the vector comprises a nucleic acid which codes for a second molecule that is linked to the reagent.

96. The reagent of claim 95, wherein the second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target.

97. The reagent of claim 96, wherein the second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target.

98. The reagent of claim 93 wherein the single chain Fv fragment is displayed on phage or wherein the Fab fragment is displayed on phage.

99. The reagent of claim 97, wherein the second molecule is a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof.

100. The reagent of claim 99, wherein the second molecule is is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant thereof which exhibits low affinity to their respective targetCD8 or anti-β_2m.

101. The reagent of claim 74, wherein the reagent is a single chain TCRVα/VL and/or mutation and variant thereof.

102. The reagent of claim 74, wherein the reagent is a single chain TCRVβ/VL and/or mutation and variant thereof.

103. The reagent of claim 74, wherein the reagent is a single chain TCRVα/VH and/or mutation and variant thereof.

104. The reagent of claim 74, wherein the reagent is a single chain TCRVβ/VH and/or mutation and variant thereof.

105. The reagent of claim 74, wherein the reagent is a single chain VL/TCRVα and/or mutation and variant thereof.

106. The reagent of claim 74, wherein the reagent is a single chain VL/TCRVβ and/or mutation and variant thereof.

107. The reagent of claim 74, wherein the reagent is a single chain VH/TCRVα and/or mutation and variant thereof.

108. The reagent of claim 74, wherein the reagent is a single chain VH/TCRVβ and/or mutation and variant thereof.

109. The reagent of claim 74, wherein the reagent is a single chain TCRVγ/VL and/or mutation and variant thereof.

110. The reagent of claim 74, wherein the reagent is a single chain TCRVδ/VL and/or mutation and variant thereof.

111. The reagent of claim 74, wherein the reagent is a single chain TCRVγ/VH and/or mutation and variant thereof.

112. The reagent of claim 74, wherein the reagent is a single chain TCRVδ/VH and/or mutation and variant thereof.

113. The reagent of claim 74, wherein the reagent is a single chain VL/TCRVγ and/or mutation and variant thereof.

114. The reagent of claim 74, wherein the reagent is a single chain VL/TCRVδ and/or mutation and variant thereof.

115. The reagent of claim 74, wherein the reagent is a single VH/TCRγ and/or mutation and variant thereof.

116. The reagent of claim 74, wherein the reagent is a single chain VH/TCRVδ and/or mutation and variant thereof.

117. A recombinant TCR recognition element reagent and mutant and /or variant thereof.

118. The reagent of claim 117, wherein the reagent is a single chain TCRVα/TCRVβ.

119. The reagent of claim 117, wherein the reagent is a single chain TCRVα/TCRVγ.

120. The reagent of claim 117, wherein the reagent is a single chain TCRVα/TCRVδ.

121. The reagent of claim 117, wherein the reagent is a single chain TCRVβ/TCRVα.

122. The reagent of claim 117, wherein the reagent is a single chain TCRVβ/TCRVγ.

123. The reagent of claim 117, wherein the reagent is a single chain TCRVβ/TCRVδ.

124. The reagent of claim 117, wherein the reagent is a single chain TCRVγ/TCRVα.

125. The reagent of claim 117, wherein the reagent is a single chain TCRVγ/TCRVβ.

126. The reagent of claim 117, wherein the reagent is a single chain TCRVγ/TCRVδ.

127. The reagent of claim 117, wherein the reagent is a single chain TCRVδ/TCRVα.

128. The reagent of claim 117, wherein the reagent is a single chain TCRVδ/TCRVβ.

129. The reagent of claim 117, wherein the reagent is a single chain TCRVδ/TCRVγ.

130. An expression vector comprising a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and polynuclotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof.

131. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVα/VL.

132. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVβ/VL.

133. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVα/VH.

134. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVβ/VH.

135. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VL/TCRVα.

136. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VL/TCRVβ.

137. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VH/TCRVα.

138. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VH/TCRVβ.